1971

The Effect of a Specific Exercise Program on the Circadian Rhythm of the Adrenal Cortex as Determined by the Urinary 17-Ketosteroids.

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DeLACERDA, Fred G., 1937-
THE EFFECT OF A SPECIFIC EXERCISE PROGRAM ON THE CIRCADIAN RHYTHM OF THE ADRENAL CORTEX AS DETERMINED BY THE URINARY 17-KETOSTEROIDS.

The Louisiana State University and Agricultural and Mechanical College, Ph.D., 1971
Education, physical

THE EFFECT OF A SPECIFIC EXERCISE PROGRAM ON THE CIRCADIAN RHYTHM OF THE ADRENAL CORTEX AS DETERMINED BY THE URINARY 17-KETOSTEROIDS

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Health, Physical, and Recreation Education

by
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August, 1971

ACKNOWLEDGMENTS

The writer wishes to express his sincere thanks to Dr. R. E. Steben for his advice and encouragement in the organization and development of this study.

The writer would also like to express his gratitude to Dr. Albert Meier for the use of his laboratory facilities. Gratitude is further expressed to Dr. Meier for his encouragement and advice concerning research in physiological rhythm.

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ABSTRACT

The primary purpose of the study was to investigate the effect of a specific exercise program on the circadian rhythm of the adrenal cortex. The concentration of 17-ketosteroid (17-KS) in the urine of six subjects served as an indicator of adrenal cortical function.

The exercise program consisted of an exercise bout on a bicycle ergonmeter conducted once a day for five consecutive days per week over a three week period. The adrenal cortical circadian pattern was determined by plotting results of urinalysis conducted four times during a twenty-four hour period. Two such twenty-four hour determinations were made each week and were used to calculate an average for that week. The week prior to the beginning of the exercise program was used to establish a control pattern.

In order to investigate the effect of the exercise program on the circadian rhythm of adrenal cortical function the circadian pattern for each week of the exercise program was graphically compared to the control pattern for each subject. The circadian pattern for the week following the exercise program was also compared to the control pattern for each subject.

The secondary purpose of the study was to determine the relationship between physical performance and the adrenal cortical circadian rhythm. Prior to the three week training

period each subject performed an exercise bout on the bicycle ergometer at 0900 hours and at 2100 hours. The duration of exercise at each exercise bout was recorded as the pre-training scores. At the end of the three week training program the same procedure was repeated with the duration of exercise being recorded as post-training scores.

A comparison was made between the 0900 hours and the 2100 hours pre-training scores to determine if peak performance was related to peak adrenal cortical function. A comparison was also made between the two post-training scores to determine if an alteration in the adrenal cortical cycle was related to a change in physical performance.

The findings of this study were as follows:

1. Three basic circadian control patterns were found among the six subjects.
2. As compared to control values, four of the subjects had an increase in 17-KS values during week one of exercise while two subjects had a decrease in 17-KS value.
3. During week two of exercise the 17-KS values for all the subjects increased as compared to the control values. At 2300 hours five of the subjects developed a secondary peak 17-KS value.
4. All of the six subjects had a primary and a secondary 17-KS peak during exercise week three.
5. The 17-KS values for the week following the exercise

program were, with the exception of one subject, elevated as compared to the control values. The 17-KS circadian patterns were parallel to the control patterns for all the subjects.

6. Prior to the training period two subjects had greater duration of exercise scores at the time of peak 17-KS values; four subjects had greater duration of exercise scores during the low point for 17-KS values.
7. Following the training program the duration of exercise scores for all subjects were greater at the 0900 hours time period than for the 2100 hours time period.

Within the limitations of this study the following conclusions were reached:

1. The exercise program utilized in this study altered the circadian pattern of the adrenal cortex as determined by the urinary 17-KS.
2. There appeared to be no relationship between physical performance and the circadian rhythm of the adrenal cortex neither before nor after the three week training program.

CHAPTER I

INTRODUCTION

There appears to be no organ nor function in the human organism which does not exhibit a similar daily rhythmicity. Whether it be the number of dividing cells in any tissue, the volume of urine excreted, the reaction to a drug, or the accuracy with which mathematical problems are solved, there is a maximum value at one time of the day and a minimum at another.[1] This daily rhythmicity is referred to as a circadian rhythm.[2]

The homeostatic concept set forth by Cannon[3] has been firmly entrenched as a physiological concept, and as a result, it has been only within the more recent years that the influence of circadian rhythms on human physiological functions has been investigated. Aerospace developments have been either largely or partly responsible for this increased interest, particularly as related to motor performance.

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[1] Jurgen Aschoff, "Circadian Rhythms in Man," Science, 148:1427-1432, 1965.

[2] F. Gerritzen, "Influence of Light on Human Circadian Rhythms," Journal of Aerospace Medicine, 37:66-70, 1966.

[3] Vernon B. Mountcastle, Medical Physiology, 12th Ed. (Saint Louis: The C. V. Mosby Company, 1968), p. 3.

Statistical observations of aircraft flights which cross several time zones revealed that the majority of people were sensitive to this phase shift. It has also been observed that athletes, actors, and even race horses were not at their physical and/or mental best the first few days after arriving from another time zone. Pilots who because of flight schedules frequently change time zones have experienced nervous disorders.[4]

Almost all life processes that occur in rhythms or cycles are endogenous, but in the course of phylogeny have become influenced by the synchronized with the external environment. For example, the physiological sleep-wake cycle is closely associated with the physical day-night cycle. In space flights the Americans and Russians have found the sleep-wake cycle to remain synchronized with the time zone to which the astronauts were adapted during the pre-launch period. Furthermore, exercise which is necessary to prevent such physiological disturbances as orthostatic hypotonia of the muscles and veins contributed to a pattern of sleep-wakefulness.[5]

The circadian rhythm of wakefulness, respiration,

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[4]Hubertus Strughold, "The Physiological Clock in Aeronautics and Astronautics," Annals of the New York Academy of Sciences, 134:417. 1965.

[5]Ibid., p. 420.

temperature, and renal function have been investigated in man. Also, the cardiovascular and hormonal systems have been studied with respect to cyclic activity.[6] However, the role of various environment stimuli on the physiological circadian rhythms in man is a much needed area of research. This is particularly true with respect to the effects of a specific exercise program on the circadian pattern of various physiological functions.

STATEMENT OF THE PROBLEM

This study was an investigation to determine if exercise would alter the circadian rhythm of the adrenal cortex in male subjects. Furthermore, since it was possible that modification of the adrenal circadian pattern could influence physical performance this investigation sought to provide information concerning the relationship of physical performance to the rhythmical pattern of adrenal cortical function.

PURPOSE OF THE STUDY

The primary purpose of the study was to investigate the effect of a specific exercise program on the circadian rhythm of adrenal cortical function and to determine any

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[6] J. N. Mills, "Human Circadian Rhythms," <u>Physiological Review</u>, 46:128-171, 1966.

alteration physical activity had on this rhythmical pattern.

A secondary purpose of this study was to investigate the relationship between physical performance and the circadian rhythm of the adrenal cortex before and after a three week exercise program.

DELIMITATIONS OF THE STUDY

The study was concerned only with the 17-ketosteroid concentration in the urine as a measure of the adrenal cortical activity. No attempt was made to differentiate the steroid output of the adrenal cortex from that of the testis and other steroidogenic tissues. However, in research evaluation the urinary 17-ketosteroids are used as an indication of adrenal cortical function.

The study was limited to a five week period of investigation.

The study was limited to male undergraduate students at Louisiana State University, Baton Rouge, Louisiana.

The collection of urine was limited to four samples during a twenty-four hour period. These twenty-four hour periods were limited to two examinations during each week of the study.

LIMITATIONS OF THE STUDY

The limitations of this study were:

1. Sleep, diet, daily habits, and sex activity of

the subjects could not be regulated.

2. The subjects were requested not to engage in any exercise program other than the one used in this study; however, there was no way to ascertain that this request was carried out.
3. The study was limited to six subjects.

SIGNIFICANCE OF THE STUDY

The exact role of adrenal cortical activity in physical performance has never been established; nevertheless, the cortical steroids are involved in electrolyte and water excretion, fat metabolism, carbohydrate metabolism, and protein metabolism. All of the above mentioned are physiological processes that directly or indirectly influence physical performance.

The existence of circadian rhythms for many physiological processes has led to the theory that an optimal time of day exists for physical performance. The various circadian rhythms may also be influenced by the time of day at which practice of a physical activity takes place. Consequently, the effect of exercise on circadian rhythms as well as the relationship between physical performance and circadian rhythms need to be investigated.

Since the adrenal cortical steroids are involved in a wide range of physiological processes and the adrenal cortical function follows a circadian pattern, it seems that the effect of an exercise program on adrenal cortical activity is in need of investigation. Furthermore, it seems that possible changes in adrenal cortical function being reflected in a person's physical performance ability is in need of study.

DEFINITION OF TERMS

Adrenal cortex. This term was defined as the outer portion of epitheliod cells of the adrenal endocrine gland that secretes the corticosteroids.[7]

Circadian rhythm. A term defined as the cycle or pattern used to include all physiological periodicity that approximate twenty-four hours.

Corticosteroids. This term was defined as the hormones arising from the adrenal cortex that have in common a chemical structure with a cyclopentano-perhydro-phenanthrene nucleus.[8]

Duration of exercise. This term was used as the period of time from the onset of an exercise bout to the time at which the heart rate of 180 beats per minute was reached.

Exercise bout. This term was defined as a training period on the bicycle ergometer set for an initial work load

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[7] C. D. Turner, *General Endocrinology*, 4th Ed. (Philadelphia: W. B. Saunders Company, 1967), p. 310.

[8] *Ibid.*, p. 312.

of zero kilo-ponds-meters/minute (kpm/min) which was progressively increased at a rate of 150 kilo-pond-meters/minute each minute, and for a work rate of eighteen kilometers per hour (km/hr) until a heart rate of 180 beats per minute was reached.

Heart rate. A term used as the number of contractions of the heart expressed as beats per minute (beats/min).

17-hydroxycorticosteroid. A term used to designate a corticosteroid with a hydroxyl group on carbon seventeen which was referred to as 17-OH-CS in this study.

17-ketosteroid. A term defined as a corticosteroid with a ketone group on carbon seventeen which was referred to as 17-KS in this study.

Time. Time was expressed on a twenty-four hour basis starting with 0100 hours and ending with 2400 hours.

Days in the week. The days in the week were numbered from one to seven with Sunday being designated as day one.

CHAPTER II

REVIEW OF RELATED LITERATURE

The presence of a circadian rhythm for adrenal cortical function in human beings was first established in 1943.[9] Since then the presence of this rhythm has been substantiated by a number of investigations. Due to the fact that the function of adrenal cortex cannot be measured directly chemical analysis is used to determine the corticosteroid levels in either the blood plasma or the urine.

Each of these methods has advantages and disadvantages. The ease of obtaining a urine sample is an obvious advantage; however, the urinary level of the corticosteroids depends upon renal function as well as upon complete emptying of the bladder for each sample. The plasma level of the cortiocosteroids is a more direct measure of adrenal function, but the stress imposed by repeated venepuncture frequently upsets the circadian rhythm.[10] Regardless of the method employed, the precision of circadian pattern identification depends on the frequency of sampling and on the particular hours chosen for sampling.

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[9]Gregory Pincus, "A Diurnal Rhythm in the Excretion of Urinary Ketosteroids by Young Men," _Journal of Clinical Endocrinology_, 3:195-199, 1943.

[10]R. I. S. Bayliss, _Ciba Foundation: Colloquia Endocrinology, The Adrenal Cortex_ (Boston: Little, Brown and Company, 1955), p. 649.

The review of related literature was limited to studies concerning circadian rhythm of adrenal cortical activity. The literature was presented under the main headings: (1) corticosteroid levels in the urine; (2) corticosteroid levels in the plasma; (3) comparison of plasma corticosteroid levels with urinary corticosteroid levels; (4) effect of exercise on adrenal cortical function.

CORTICOSTEROID LEVELS IN THE URINE

The first study to establish a circadian rhythm in adrenal cortical function was conducted by Pincus.[11] Urine specimens were collected from seven young men at six hour intervals over a twenty-four hour period and analyzed for 17-ketosteroids (17-KS). This procedure was repeated a total of forty-eight times. The night values (period of sleep) for the urinary 17-KS were regularly lower than the day values (period of wakefulness). The maximum values occurred during the day with the peak being during the late morning hours. Each individual tended to have a characteristic level of 17-KS excretion over a twenty-four hour period.

Similar results were obtained by Doe, Venner, and

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[11] Pincus, loc. cit., pp. 195-199.

Flink.[12] In this case, however, the 17-hydroxycorticosteroid (17-OH-CS) concentration in the urine of eight male subjects was determined every three hours for twenty-four hours. A peak was found at 1100 hours and a low at 0300 hours.

Bartter, Delea, and Halberg[13] investigated the levels of both 17-KS and 17-OH-CS in the urine of seven college girls with samples taken every three hours for a total of thirty hours. The 17-KS and the 17-OH-CS followed parallel patterns with the peak values being between 1200 and 1500 hours which was approximately six hours after awakening. The low values were found to be between 2400 and 0300 hours.

Sharp, Slorach, and Vipond[14] investigated the circadian rhythm of the adrenal cortex after complete reversal of the sleep-activity pattern. During a twenty-four hour control period urine samples were collected

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[12]Richard P. Doe, J. A. Venner, and E. B. Flink, "Diurnal Variation of 17-Hydroxycorticosteroids, Sodium, Potassium, Magnesium, and Creatinine in Normal Subjects and in Cases of Treated Adrenal Insufficiency and Cushing's Syndrome," Journal of Clinical Endocrinology and Metabolism, 20: 253-265, 1960.

[13]F. C. Bartter, C. S. Delea, and Fritz Halberg, "A Map of Blood and Urinary Changes Related to Circadian Variations in Adrenal Cortical Function in Normal Subjects," Annals of the New York Academy of Sciences, 98:969-983, 1962.

[14]G. W. G. Sharp, S. A. Slorach, and H. J. Vipond, "Diurnal Rhythms of Keto- and Ketogenic Steroid Excretion and the Adaptation to Changes of the Activity-Sleep Routine," Journal of Endocrinology, 22:377-385, 1961.

every three hours and analyzed for 17-KS. Following the control period there was a twelve hour reversal of the sleep-activity cycle for a period of eight days. Every two days during this reversal the urine was analyzed at three hour intervals for 17-KS. After the reversal period the subjects reverted to a normal sleep-activity pattern that was followed by eight days of testing the levels of 17-KS in the urine.

During the control period the 17-KS output was higher by day than by night, with the peak value being in the late morning hours. There was a reversal of this rhythm on the second day after the sleep-activity change. This pattern persisted throughout the eight day testing period. However, the 17-KS pattern returned to normal the second day after resumption of the normal sleep-activity routine.

The effect of sudden change in geographic position on the periodicity of adrenal cortical function was investigated by Flink and Doe.[15] The study was conducted with one subject on an airplane trip from Minneapolis, Minnesota, to Seoul, Korea. At each of five different test periods during the flight and upon arrival in Korea urine samples were collected every three hours for a thirty hour period and analyzed for 17-OH-CS.

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[15]E. B. Flink and R. P. Doe, "Effect of Sudden Time Displacement by Air Travel on Synchronization of Adrenal Function," Society for Experimental Biology and Medicine, 100:498-501, 1959.

The peak values at Minnesota occurred from 0600 to 1200 hours. On arrival in Korea the pattern was synchronized with Minnesota Central Standard Time (CST); however, after nine days in Korea the 17-OH-CS pattern became synchronized with the Korean time schedule. The Korean time schedule was displaced 9.5 hours from the CST of Minnesota. The 17-OH-CS rhythm was found to have shifted approximately nine hours, but had not achieved the normal amplitude after two months. The sleep cycle required about three weeks to adapt to the new time schedule.

CORTICOSTEROID LEVELS IN THE PLASMA

The existence of a circadian variation for the plasma 17-OH-CS has been reported by several authors. With sampling every four hours the peak value was found at 0800 hours in ten subjects of one study,[16] in seven subjects of a second study,[17] and in six subjects of another study.[18]

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[16]H. Brown, E. Englert, S. Wallach, and E. L. Simons, "Metabolism of Free and Conjugated 17-Hydroxycorticosteroids in Normal Subjects," Journal of Clinical Endocrinology and Metabolism, 17:1191-1201, 1957.

[17]C. A. Nugent, K. Eik-Nes, H. S. Kent, L. T. Samuels, and F. H. Tyler, "A Possible Explanation for Cushing's Syndrome Associated with Adrenal Hyperplasia," Journal of Clinical Endocrinology and Metabolism, 20:1259-1268, 1960.

[18]R. E. Peterson, "Plasma Corticosterone and Hydroxycortisone Levels in Man," Journal of Clinical Endocrinology and Metabolism, 17:1150-1157, 1957.

Continuous determinations of plasma 17-OH-CS values in five subjects over a four day period showed a constant repetition of the same pattern.[19] Sampling every three hours Doe, Venner, and Flink[20] found the peak values to be between 0600 and 0900 hours.

In the studies cited to this point the subjects were ambulatory by day and asleep at night. In order to define possible factors accounting for the periodicity of the plasma 17-OH-CS Migeon[21] and others collected blood samples every four hours from twelve control subjects, ten night workers, and six blind subjects. The control group had a rhythm of the plasma 17-OH-CS with peak values at 0800 hours and low values at 2400 hours. Night workers and blind subjects were found to have the same rhythm as the control subjects; however, it was noted that the night workers had been on night duty at least six months prior to the experiment.

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[19] G. T. Perkoff, K. Eik-Nes, C. A. Nugent, H. L. Fred, R. A. Nimer, L. Rush, L. T. Samuels, and F. H. Tyler, "Studies of the Diurnal Variation of Plasma 17-Hydroxycorticosteroids in Man," Journal of Clinical Endocrinology and Metabolism, 19:432-443, 1959.

[20] Doe, Venner, and Flink, loc. cit., pp. 253-265.

[21] C. J. Migeon, F. H. Tyler, J. P. Mahoney, A.A. Florentin, H. Castle, E. L. Bliss, and L. T. Samuels, "The Diurnal Variation of Plasma Levels and Urinary Excretion of 17-Hydroxycorticosteroids in Normal Subjects, Night Workers, and Blind Subjects," Journal of Clinical Endocrinology and Metabolism, 16:622-633, 1956.

Eight adults were subjected to reversal of their night sleep and work patterns in a study by Perkoff.[22] In contrast to the findings of the previous study, the pattern of the plasma 17-OH-CS concentration showed a distinct inversion by the end of the reversal period. As early as the fourth day in some subjects and as late as the eighth day in others, the 0800 hour plasma values decreased markedly.

Two groups of male subjects were employed in a study by Halberg[23] and others. One group followed their normal wake-sleep cycle. During the day they followed the usual work pattern as staff technicians in a hospital. The second group followed their regular work pattern as technicians continuously for forty-eight hours without any period of sleep. The plasma 17-OH-CS level was determined every six hours for forty-eight hours for each subject.

Both groups had 17-OH-CS patterns which peaked from 0600 to 1200 hours with the low values at 2400 hours; however, in the case of the continuously active group the circadian cycle was enhanced so the peak-to-through difference was greater than that of the normal group.

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[22]Perkoff and others, loc. cit., pp. 432-443.

[23]F. Halberg, G. Frank, R. Harner, J. Matthews, H. Aaker, H. Gravem, and J. Melby, "The Adrenal Cycle in Men on Different Schedules of Motor and Mental Activity," Experientia 17:282-284, 1961.

There is evidence to suggest that the adrenal rhythm depends on high level activity of the central nervous system. For example, the circadian rhythm of the plasma 17-OH-CS completely disappeared in a man who spent three months alone underground, even though he followed a regular circadian cycle of activity, meals, and sleep.[24] In cases of patients suffering from Cushing's syndrome there was an absence of a plasma corticosteroid rhythm.[25]

An investigation by Eik-Nes and Clark[26] was based on the assumption that processes in the central nervous system are involved in the awareness of rhythmic changes in the external environment which in turn gives rise to a circadian pattern of the plasma 17-OH-CS. To test this hypothesis four subjects were studied all having severe central nervous system disorders involving a profound degree of impairment of consciousness. Two normal subjects served as controls.

In the four brain damaged subjects there were erratic changes in the plasma 17-OH-CS levels, but with no consistent

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[24]J. N. Mills, "Circadian Rhythms During and After Three Months in Solitude Underground," Journal of Physiology (London), 174:217-231, 1964.

[25]Doe, Venner, and Flink, loc. cit., pp. 253-265.

[26]Kristen Eik-Nes and L. D. Clark, "Diurnal Variation of Plasma 17-Hydroxycorticosteroids in Subjects Suffering from Severe Brain Damage," Journal of Clinical Endocrinology and Metabolism, 18:764-768, 1958.

relationship to day-night. The normal subjects showed a reproducible pattern of the plasma 17-OH-CS levels. Thus, it seemed that serious disorders of the central nervous system associated with loss of awareness of the external environment impaired the normal circadian rhythm of the adrenal cortex.

After review of a wide range of research evidence, Mills[27] concluded that of the many periodic stimuli that impinge upon the central nervous system, the most important of these is social contact with other people whose activity and sleep habits follow a circadian rhythm. This periodic excitation of the central nervous system results in a rhythmical release of corticotrophic-releasing-factor (CRF) from the region of the median eminence. The rate of synthesis and release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary depends on the action of CRF. This periodic release of ACTH results in periodic activity of the adrenal cortex. This in turn is evidenced by the circadian variation in the plasma concentration and urinary excretion of the corticosteroids.

COMPARISON OF PLASMA CORTICOSTEROID LEVELS WITH URINARY CORTICOSTEROID LEVELS

The plasma levels of the free corticosteroids are a

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[27] J. N. Mills, "Human Circadian Rhythms," Physiological Review, 46:128-171, 1966.

more direct measure of the hormonal environment within the living organism. The rate of the urinary excretion of the conjugated corticosteroids is only an indirect reflection of this environment since it is delayed by glomerular filtration, and it involves a series of intermediate reactions whereby the hormone is converted into inactive compounds. However, since repeated venepuncture required for plasma determinations upsets the rhythm of the adrenal cortex, a stress factor nearly eliminated by urine sampling, the relationship between plasma and urinary corticosteroid levels is of considerable interest in the study of rhythmic function of the adrenal cortex.[28]

One study[29] investigated the twenty-four hour variation in the levels of free and of conjugated 17-OH-CS in the plasma of ten subjects. The level of the free 17-OH-CS was highest between 0600 and 0800 hours and was lowest between 2400 and 0400 hours. The levels of conjugated 17-OH-CS followed the same pattern but lagged behind by a period of two to four hours.

An investigation[30] of the circadian rhythm for the 17-OH-CS levels in the plasma and in the urine was conducted on

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[28]Mills, loc. cit., p. 141.

[29]Brown and others, loc. cit., pp. 1191-1201.

[30]Doe, Venner, and Flink, loc. cit., pp. 253-265.

eight subjects. With collection of samples every three hours, the variation in the urinary 17-OH-CS level was found to follow the plasma fluctuations closely; however, the peak urinary 17-OH-CS level lagged behind the peak plasma level by approximately three hours. Comparable results were obtained in another study where the plasma and urine samples were analyzed every four hours for the 17-OH-CS.[31]

A study by Bartter, Delea, and Halberg[32] compared the urinary 17-KS levels with the 17-OH-CS plasma levels in seven college girls. The urinary 17-KS levels and the plasma levels of the 17-OH-CS followed a parallel pattern, but again, the urinary levels lagged behind that of the plasm levels by two to three hours.

EFFECT OF EXERCISE ON ADRENAL CORTICAL FUNCTION

Research concerning the effect of exercise on adrenal cortical activity has been largely concerned with adaptation to stress. For example, Girardin[33] was concerned with human adaptation to stress as measured by the 17-KS level

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[31]Migeon and others, loc. cit., pp. 622-633.

[32]Bartter, Delea, and Halberg, loc. cit., pp. 969-983.

[33]Yvan Girardin, "The Comparative Effects of Two Levels of Training Intensity on Human Adaptation to a State of Stress" (Unpublished Dissertation, Florida State University, Tallahassee, Florida, 1967).

in the urine when the subjects were exposed to exercise bouts of varying intensity. This 17-KS level was a single determination on a total urine sample collected for twenty-four hours. Jovey and others[34] observed the changes in plasma 17-OH-CS level when a group of subjects were exposed to exercise. The plasma samples were collected only once in a twenty-four hour period. In neither of these two studies was any thought given to the circadian pattern of adrenal cortical activity.

Despite investigation into the effects of the sleep-activity cycle on the circadian function of the adrenal cortex, little research has dealt with the effects of a specific exercise program on this circadian pattern. The purpose of a study by Vallbona and others[35] was to find out whether continuous bedrest had an effect on the circadian rhythm of the 17-OH-CS in the plasma of six subjects and to detect the possible effect of isometric exercise on this rhythm.

Plasma levels of 17-OH-CS were determined every four

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[34]D. Jovy, H. Bruner, K. E. Klein, and H. M. Wegmann, "Adaptive Responses of Adrenal Cortex to Some Environmental Stressors, Exercise, and Acceleration," Hormonal Steroids, Biochemistry, Pharmacology and Therapeutics, Proceedings of the First International Congress on Hormonal Steroids, 2:545-553, 1965.

[35]Carlos Vallbona and others, "Influence of Bedrest on Plasma Levels of 17-Hydroxycorticosteroids," Journal of Aerospace Medicine, 36:524-528, 1965.

hours for twenty-four hours while the subjects were ambulatory and during two three day periods of bedrest. During the first period the subjects were at bedrest only. During the second period a program of isometric exercise was added to the bedrest.

The plasma 17-OH-CS pattern consisted of a 0800 peak followed by a progressive decrease until a low was reached at midnight. During the bedrest period the peak value at 0800 seemed lower than the peak value when the subjects were ambulatory; however, analysis of variance failed to show any significant difference between ambulatory and bedrest conditions. Also, the exercise routine failed to alter the circadian pattern found during bedrest.

SUMMARY OF RELATED LITERATURE

The existence of a circadian rhythm for adrenal cortical activity has been well established through the investigation of the 17-KS and the 17-OH-CS concentrations in both the plasma and the urine. Peak values for the corticosteroids occurred during the late morning hours with the low values being during the early morning hours.[36, 37,38,]

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[36]Pincus, loc. cit., pp. 195-199.

[37]Doe and others, loc. cit., pp. 253-265.

[38]Bartter and others, loc. cit., pp. 969-983.

[39, 40, 41, 42] Corticosteroid levels in the urine followed a parallel pattern with those in the plasma; however, the urinary peak lagged behind the plasma peak by two to three hours.[43, 44, 45, 46]

The exact relationship of adrenal activity to the sleep-activity cycle remains uncertain. Reversal of the sleep-activity cycle resulted in the inversion of the adrenal cortical circadian pattern. Returning to a normal sleep-activity cycle, the adrenal secretory pattern reverted to normal timing.[47] However, this reversal in adrenal activity was not found in blind subjects or in night workers.[48] A change in time zones by 9.5 hours resulted in a phase shift of corticosteroid excretion by nine hours.[49]

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[39]Brown and others, loc. cit., pp. 1191-1201.

[40]Nugent and others, loc. cit., pp. 1259-1268.

[41]Peterson, loc. cit., pp. 1150-1157.

[42]Perkoff and others, loc. cit., pp. 432-443.

[43]Brown and others, loc. cit., pp. 1191-1201.

[44]Doe and others, loc. cit., pp. 253-265.

[45]Migeon and others, loc. cit., pp. 622-633.

[46]Bartter and others, loc. cit., pp. 969-983.

[47]Sharp and others, loc. cit., pp. 377-385.

[48]Migeon and others, loc. cit., pp. 622-633.

[49]Flink and Doe, loc. cit., pp. 498-501.

There seems to be central nervous system involvement in the circadian activity of the adrenal cortex. An absence of circadian rhythm existed in brain damaged subjects who had impairment of consciousness.[50] Absence of an adrenal rhythm was found in Cushing's disease, a condition involving deranged function of the pituitary gland.[51]

The effect of specific exercise programs on rhythmical adrenal activity has been subjected to sparse investigation. An isometric exercise program imposed upon subjects in a bedrested condition failed to alter the circadian pattern of the adrenal cortex.[52] However, continuous activity as lab technicians over a forty-eight hour period enhanced the circadian pattern of the adrenal cortex.[53]

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[50]Kristen and Clark, loc. cit., pp. 764-768.

[51]Doe and others, loc. cit., pp. 253-265.

[52]Vallbona and others, loc. cit., pp. 524-528.

[53]Halberg and others, loc. cit., pp. 282-284.

CHAPTER III

OVERVIEW OF PROCEDURE

The effect of a specific exercise program on the circadian rhythm of the adrenal cortex was studied utilizing six male students as subjects. The concentration of the 17-KS in the urine served as an indicator of adrenal cortical function.

The exercise program consisted of an exercise bout on a bicycle ergometer. These bouts were conducted once a day for five consecutive days per week over a three week period. At each exercise bout the work load on the ergometer was increased 150 kilo-pond-meters per minute each minute of exercise until the heart rate reached 180 beats per minute.

The circadian pattern was determined by plotting results of urinalysis conducted four times during a twenty-four hour period. Two such twenty-four hour determinations were made each week. From these determinations an average was calculated for each week. The week prior to the beginning of the exercise program was used to establish a control pattern.

In order to investigate the effect of the exercise program on the circadian rhythm of adrenal cortical function the circadian pattern for each week of the exercise program was graphically compared to the control pattern for each subject. The circadian pattern for the week following the exercise program was also compared to the control pattern for each subject.

In order to determine the relationship between physical performance and the adrenal cortical circadian rhythm each subject performed an exercise bout on the bicycle ergometer at 0900 hours and at 2100 hours prior to the three week training period. The times 0900 hours and 2100 hours were selected for the exercise bout since the former approximated peak adrenal cortical function and the latter approximated the low point in adrenal cortical activity. The duration of exercise at each exercise bout was recorded in minutes as the pre-training scores. At the end of the three week training program the same procedure was repeated with the duration of exercise being recorded as post-training scores.

A comparison was made between the 0900 hours and the 2100 hours pre-training scores to determine if peak performance was related to peak adrenal cortical function. A comparison was also made between the two post-training scores to determine if an alteration in the adrenal cortical cycle was related to a change in physical performance.

SUBJECTS

The subjects in this study were six male undergraduate students at Louisiana State University, Baton Rouge, Louisiana, selected from a group of eleven volunteer students. Selection was based on availability of the subjects at the time required for the study and on the subjects being free from participation in any physical exercise program other than the one used in this study. The subjects selected were paid for participation in the study.

EQUIPMENT

E and M Solid Wire Cardiotachometer.[54] The E and M cardiotachometer system was used to determine heart rates In this system, operated by means of surface electrodes attached by adhesive washers and electrode conducting paste to the chest, the cardiac impulse was fed into a cardiac preamplifier to increase the signal to a level appropriate for the Transducer-Monitor-Coupler. The Transducer-Monitor-Coupler served to regulate an output signal suitable for input into the cardiotachometer. The cardiotachometer displayed the average heart rate on a scale with a range from zero to 250 beats per minute.

Bicycle Ergometer.[55] The Monarch bicycle ergometer

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[54]Instruction Manual, E and M Solid Wire Cardiotachometer. E and M Instrument Company, Inc., Houston, Texas.

[55]Instruction Manual, Ergometry: Test of Physical Fitness. AB Cykelfabriken Monark, Varberg, Sweden.

was used as a means of exercising the subjects. The Monark bicycle ergometer allowed for the adjustment of work loads from zero to seven kiloponds. At a rate of fifty complete pedal turns per minute the rate of work in kilopond-meters per minute could be obtained. Prior to the study it was found that a speedometer reading of eighteen kilometers per hour corresponded to a rate of fifty revolutions per minute.

Bausch and Lomb Spectronic 20.[56] The Spectronic 20 spectrophotometer was used to estimate the quantity of 17-KS in the urine. The instrument operated by passing a monochromatic light through a colorimeter cell containing the sample. Part of the beam of light was absorbed by components of the sample with the remaining light collected by a phototube where light energy was converted to an electrical signal which was expressed as optical density and percent transmission.

URINARY 17-KETOSTEROID ANALYSIS

Collection of Urine Samples. Urine samples were collected on the second and fourth days of the week prior to the exercise program, during the three weeks of the

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[56] Arnold Dunn and Joseph Arditti, Experimental Physiology, (Dallas: Holt, Rinehart, and Winston, Inc., 1968), pp. 194-208.

exercise program, and during the week following this exercise program. At 2300 hours the bladder was completely emptied and the urine discarded. All urine excreted from this time until 0730 hours was placed in a plastic container. The bladder was completely emptied at 0730 hours. All urine from this time until 1200 hours was placed in a second plastic container with the bladder being completely emptied at 1200 hours. This procedure was followed from 1200 to 1700 hours and from 1700 to 2300 hours.

At the end of each collection period the total volume of urine excreted for that time period was determined and recorded. A twenty-five milliliter sample was refrigerated and analyzed the following day for 17-KS.

Principle of Analysis for 17-KS. The urine contains a variation of steroidal compounds which may be divided into three general types: acidic, phenolic, and neutral. The neutral steroids, referred to as the 17-ketosteroids, reflect the excretion of metabolic derivatives from the secretions of the gonads and the adrenal cortex. The 17-KS appear in the urine in non-active conjugated forms and must be freed by hydrolysis and separated from the acidic and phenolic steroids. The residue so obtained was dissolved in alcohol and treated with m-dinitrobenzene and potassium hydroxide. A violet-purple color was produced which was compared with the amount of color produced by a known amount of dehydroisoandrosterone.[57]

Procedure for 17-KS Analysis. The procedure employed to analyze the urine for 17-KS was a modification of that used by the Special Chemistry Laboratory at Baton Rouge General Hospital, Baton Rouge, Louisiana.

The procedures for the extraction of the 17-KS were as follows:

1. Five milliliters of centrifuged urine from each sample were placed into four different test tubes. Five milliliters of distilled water were added to each tube for a times two dilution. In a fifth test tube a times two dilution of a commercially prepared Urine Chemistry Control was made.
2. One milliliter of Hydrolysis Sulfuric (sixty-nine percent sulfuric acid) was added to each tube and mixed by swirling.
3. The tubes were capped with perforated caps and placed in a boiling water bath for ten minutes.
4. The tubes were removed and allowed to cool to room temperature.
5. The cooled specimens were placed in separatory funnels. At least fifteen milliliters of refrigerated anhydrous ether were added to each funnel.

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[57] H. A. Harper, Review of Physiological Chemistry, 9th Ed. (Los Altos, California: Lange Medical Publications, 1963), p. 375.

Ether was used to wash each tube after pouring the urine mixture into the funnels.

6. The funnels were capped and swirled gently for three minutes each in order to extract the 17-KS.
7. The layers were allowed to separate and the bottom layer was then removed.
8. The ether layer was washed with fifteen percent sodium hydroxide four times or until no chromogen color was visible in the bottom layer. About ten milliliters was used for each washing.
9. The ether layer was washed three times with demineralized water, the last wash being checked with pH paper. The pH was to be neutral or acidic. If it was alkaline the wash was repeated and the pH rechecked.
10. The bottom layer of water was fully drained and the ether layer was then poured through the top of the funnel. The ether was passed through filter paper and into dry test tubes. No water was allowed to get into the test tubes.
11. The test tubes with ether were placed in a water bath and allowed to evaporate fully to dryness.

The concentration of 17-KS extracted was estimated colorimetrically by means of the Zimmerman reaction. This procedure was as follows:

1. In addition to the five test tubes containing the extracted 17-KS two tubes were marked as Blank and Standard.
2. To the Blank and five test tubes with extracted 17-KS were added 0.5 milliliters of purified methanol. All of the residue in the test tubes was dissolved.
3. To the Standard was added 0.5 milliliters of a known concentration of dehydroisoandrosterone. The concentration was 7.5 milligrams per liter.
4. To all the tubes were added 0.5 milliliters of aqueous dinitrobenzene.
5. To all the tubes were added 0.5 milliliters of five normal (5N) potassium hydroxide. The contents were mixed by swirling. The tubes were allowed to stand at room temperature for ten minutes.
6. Five milliliters of amyl acetate were added to each tube. All the color was removed from the potassium hydroxide by shaking the tube.
7. The amyl acetate layer was transferred to colorimeter cells.
8. The Bausch and Lomb Spectronic 20 was used to determine the optical density (OD). The wavelength was set at 530 mu and standardized to 100 percent transmission with the Blank. The optical

density of Standard and unknowns were determined.

9. Calculations:

$$\frac{\text{OD of unknown}}{\text{OD of Standard}} \times \frac{\text{Volume of urine in the sample}}{\text{Number of hours to collect sample}}$$

$$\times \text{ Dilution factor } \times \text{ Concentration of Standard}$$

$$= \text{Milligrams of 17-KS per hour}$$

All of the above chemical procedures were performed under a ventilation hood.

EXERCISE PROCEDURE

Pre-exercise conditions. The subjects exercised at scheduled time intervals during the time period from 2100 to 2300 hours five consecutive days per week. This time interval for exercise bouts was approximately ten to twelve hours following the peak circadian values of the 17-KS in the urine. The room temperature was maintained at seventy to seventy-two degrees Fahrenheit. The cardiotachometer system was properly calibrated prior to the arrival of the subjects.

Prior to the exercise bout the subjects reclined on a bed for five minutes to allow the heart rate to stabilize. While in the supine position three electrodes were attached to the subject by means of adhesive washers and conducting paste. One electrode was placed on the upper third of the sternum, a second just below the left nipple, and a third on the fifth rib at the mid-axillary line.

Exercise bout. Following the five minute rest period and the attachment of the electrodes, the subject mounted the bicycle ergometer. The electrode leads were attached to the cardiac preamplifier.

Upon the command "Go" the subject began to pedal the bicycle ergometer. The bicycle was pedaled at a work rate of fifty revolutions per minute. This rate was kept constant by the subjects observing a speedometer on the bicycle ergometer. When the work rate reached the desired level a chronometer with a sixty second sweephand was started.

The work load on the bicycle ergometer was zero kilo-pond-meters per minute for the first minute. At the end of each minute of exercise the work load was increased by 150 kilo-pond-meters per minute. This procedure was repeated until a heart rate of 180 beats per minute was registered on the cardiotachometer. When the heart rate reached the desired level the command "Stop" was given. The electrodes were removed from the subject and the next subject was then prepared for the exercise.

The exercise program was conducted three weeks with the subjects exercising on the bicycle ergometer day one through day five of each week.

Physical performance and adrenal cortical rhythm. In order to determine the relationship between physical performance and the adrenal cortical circadian rhythm each subject performed an exercise bout on the bicycle ergometer during the time period from 0900 to 1100 hours and again during the period from 2100 to 2300 hours. The time period 0900 to 1100 hours approximated the peak adrenal cortical function. The time period 2100 to 2300 hours approximated the low point in adrenal cortical activity.

The exercise bouts were conducted on day five and day six of the control week. On day five, three subjects performed during the 0900 to 1100 hours period while the remaining three subjects performed during the 2100 to 2300 hours period. This procedure was reversed on day six. The duration of exercise at each exercise bout was recorded in minutes as the pre-training scores.

On day five and day six of the third week of exercise this same procedure was repeated with the duration of each exercise bout being recorded as post-training scores.

PRESENTATION OF DATA

The concentration of 17-KS in the urine was determined four times in a twenty-four hour period, the times being at 0730 hours, 1200 hours, 1700 hours, and 2300 hours. Two twenty-four hour determinations were made during each week of the study with the determinations being made on day two and day four of each week. A weekly average expressed as milligrams per hour of 17-KS was calculated for each of the four values in a twenty-four hour period. The total twenty-four excretion of 17-KS was determined from these average values. This procedure was followed for each subject during the five week study.

To investigate the effect of the exercise program on the circadian rhythm on adrenal cortical function and to determine any alteration of this rhythmical pattern, four graphs were plotted for each subject. One graph was for each week of the exercise program and one graph was for the week following the exercise program. Also, plotted on each of the four graphs were the average 17-KS values for the week prior to the exercise program. Thus, a graphical comparison between the circadian pattern for each week of exercise and the follow-up week was made with the circadian pattern for the week prior to the exercise program.

In order to determine the relationship between physical performance and the adrenal cortical circadian rhythm, a comparison was made between the pre-training scores and the 17-KS values to determine if peak performance was related to peak adrenal cortical function. A comparison was also made between post-training scores and the 17-KS values to determine if an alteration in the adrenal cortical cycle was related to a change in physical performance.

CHAPTER IV

PRESENTATION AND ANALYSIS OF DATA

Graphical analysis was employed to study the effect of exercise on the circadian rhythm of the adrenal cortex. Comparisons were made between duration of exercise scores to determine if the circadian pattern of the adrenal cortex influenced an individual's ability to perform on the bicycle ergometer. The data collected in this study are presented in the Appendix.

GRAPHICAL ANALYSIS OF 17-KS VALUES

Control patterns for adrenal cortical function. The control patterns for the six subjects were represented by the solid line graphs in Figures 1 through 6. Three basic circadian patterns were found among the six subjects in this study. Subjects A, B, E, and F had peak 17-KS values only at 1200 hours. There was a steady decline from this peak until either 2300 or 0700 hours at which time the 17-KS values began to rise. Subjects C and D had peak 17-KS values at 1200 hours; in addition, a secondary peak of lesser magnitude was found at 2300 hours.

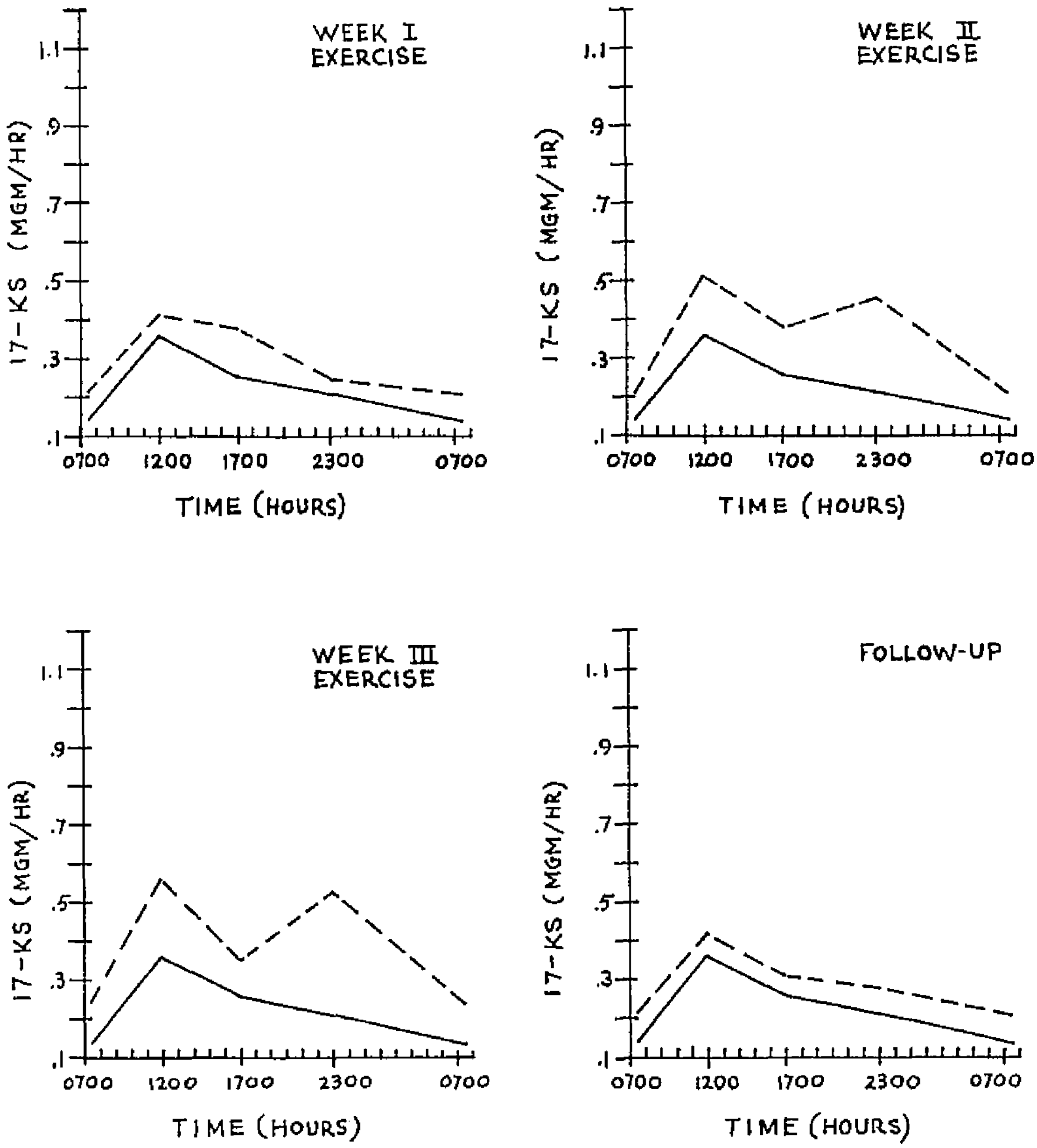
Experimental patterns for adrenal cortical function. The experimental 17-KS patterns for the six subjects in this study are represented by dashed line graphs in Figure 1 through 6.

For week one of exercise the 17-KS values of subjects A, B, C, and F increased in comparison to the control values, the largest increase being at 1700 hours. Subjects D and E had a decrease in 17-KS values; however, the 17-KS value was higher than the control value at 2300 hours for subject E.

During week two of exercise the 17-KS values of all subjects increased as compared with the control values. In the case of subject D the 17-KS values were elevated except for the 1200 hours value which was lower than the control value for that time. At 2300 hours subjects A, B, C, D and F developed a secondary peak 17-KS value. The 2300 hours 17-KS value for subject E was elevated but to a lesser degree than the value at 1700 hours.

All of the six subjects revealed a primary and a secondary peak during exercise week three. The entire circadian pattern was elevated for subjects A, B, C, E, and F. The primary peak was at 1200 hours with the secondary peak being at 2300 hours. The 17-KS values at 1200 and 1700 hours were below the control values for subject D. The 17-KS values at 2300 and 0700 hours were elevated as compared to the control values. The 1700 hours value was the low point for this subject.

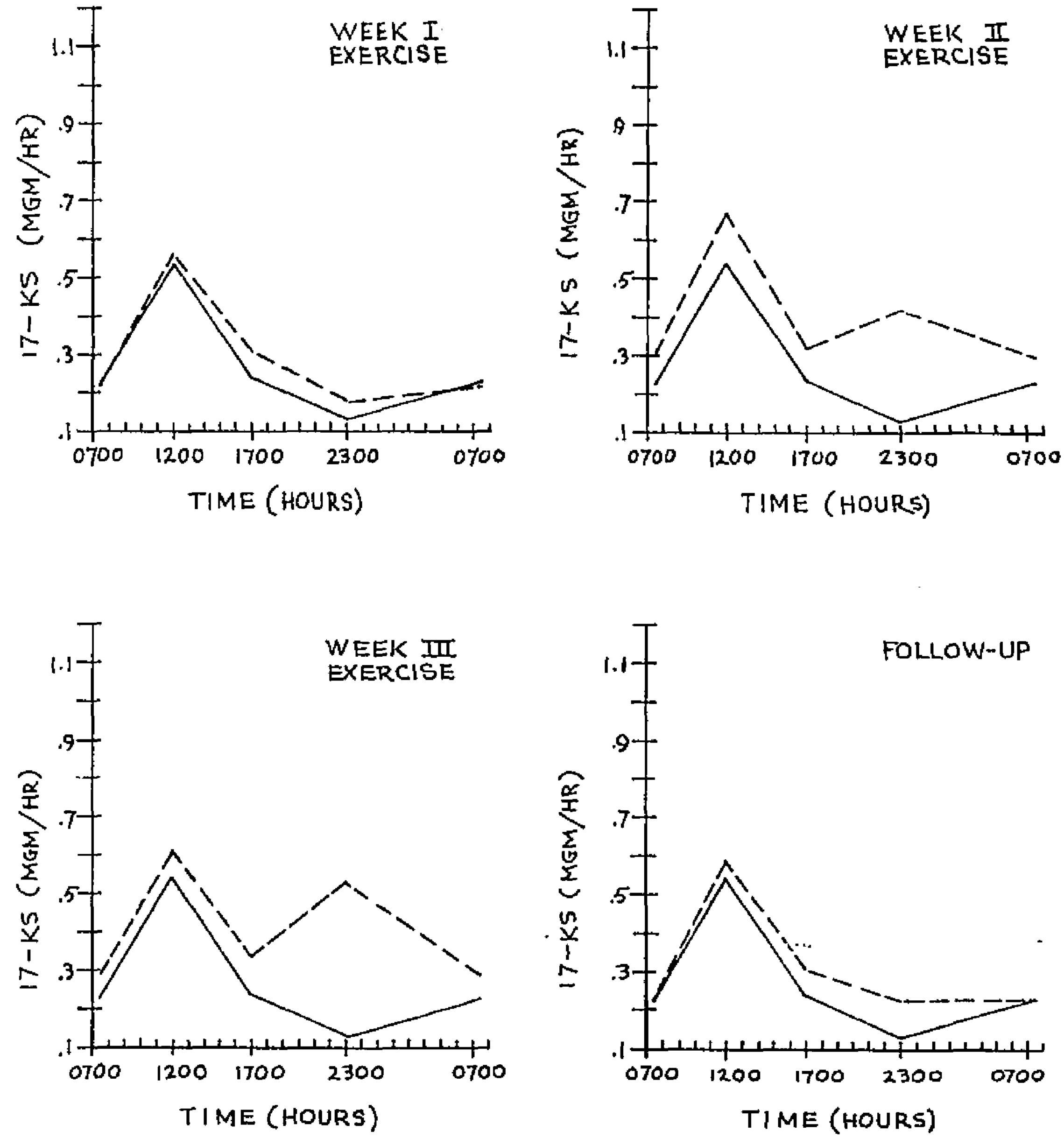
The 17-KS values for the week following the exercise



Control: ———— Experimental: - - - -

FIGURE 1

CIRCADIAN VARIATION IN 17-KS VALUES
BEFORE, DURING, AND AFTER A THREE WEEK
TRAINING PROGRAM FOR SUBJECT A

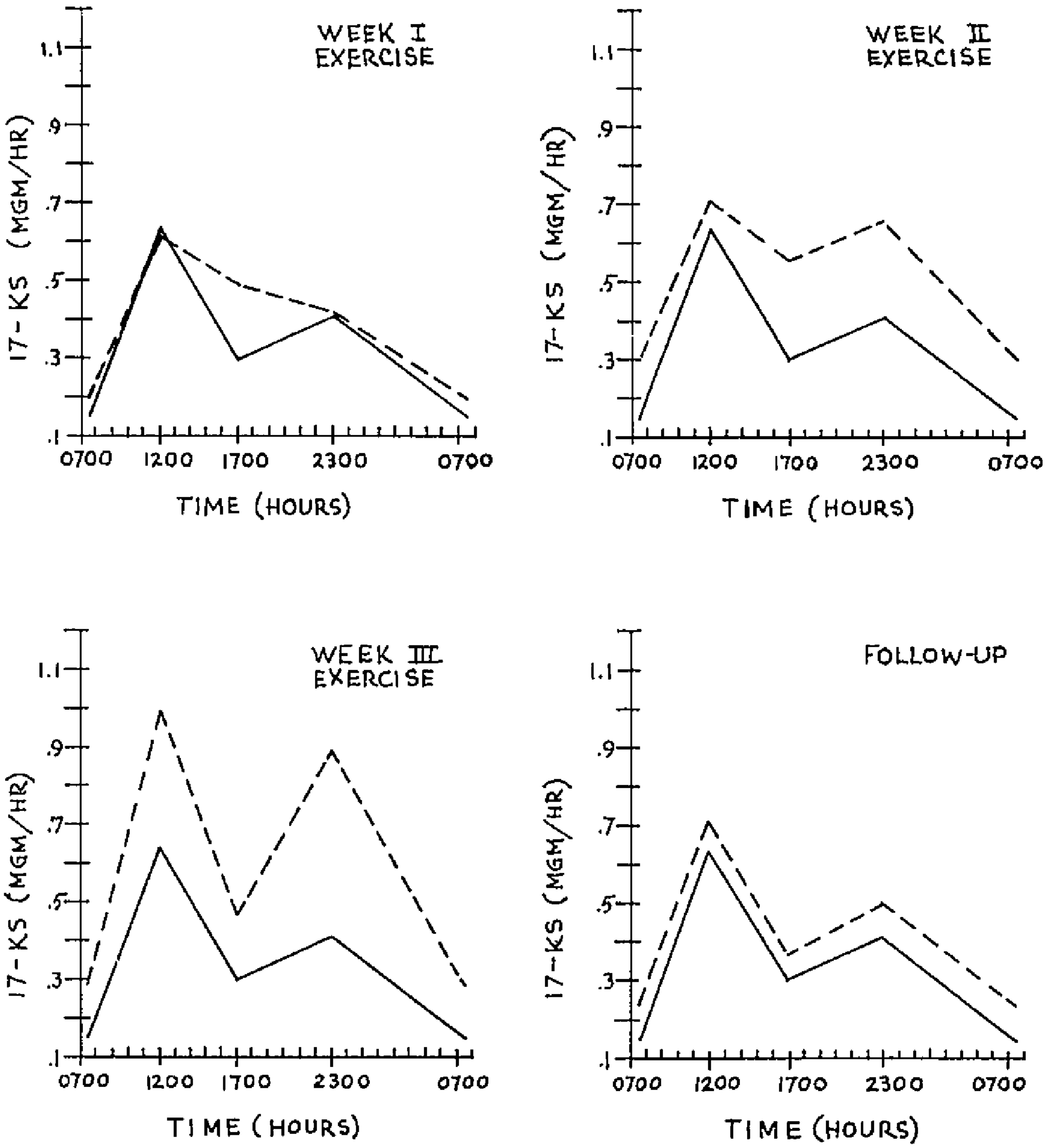


Control: ———

Experimental: - - - -

FIGURE 2

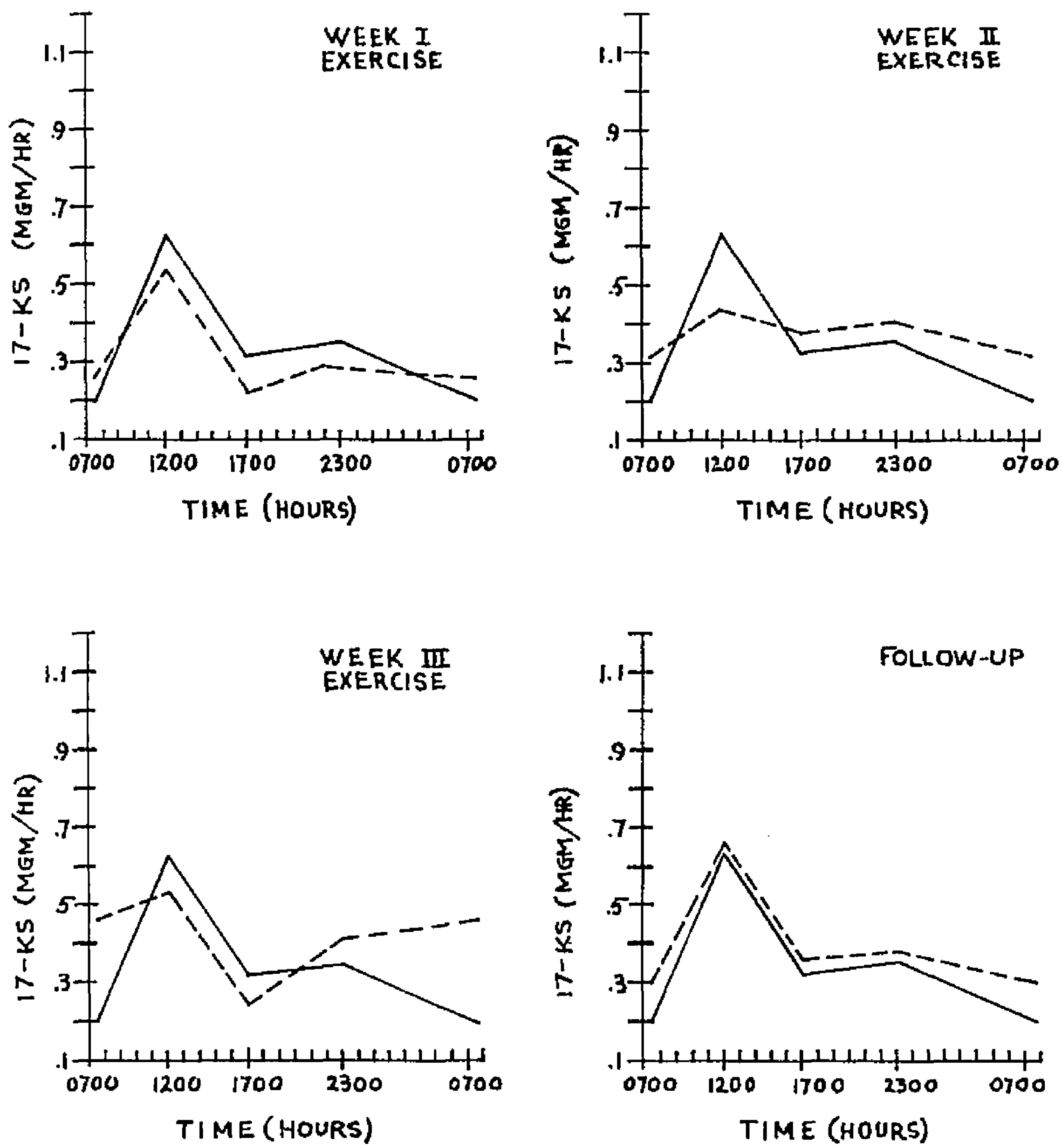
CIRCADIAN VARIATION IN 17-KS VALUES BEFORE, DURING, AND AFTER A THREE WEEK TRAINING PROGRAM FOR SUBJECT B



Control: ———— Experimental: - - - -

FIGURE 3

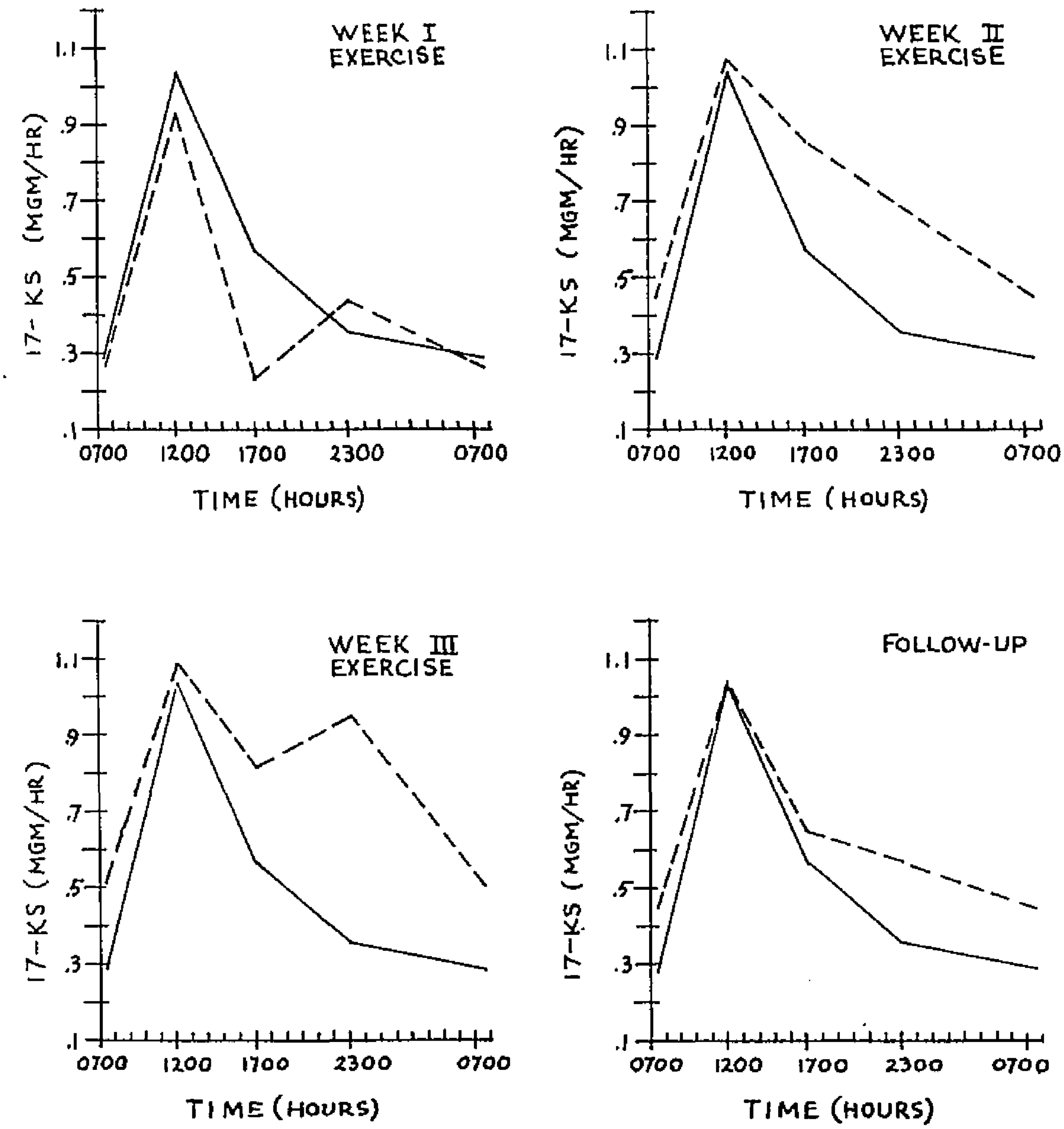
CIRCADIAN VARIATION IN 17-KS VALUES
BEFORE, DURING, AND AFTER A THREE WEEK
TRAINING PROGRAM FOR SUBJECT C



Control: ———— Experimental: - - - -

FIGURE 4

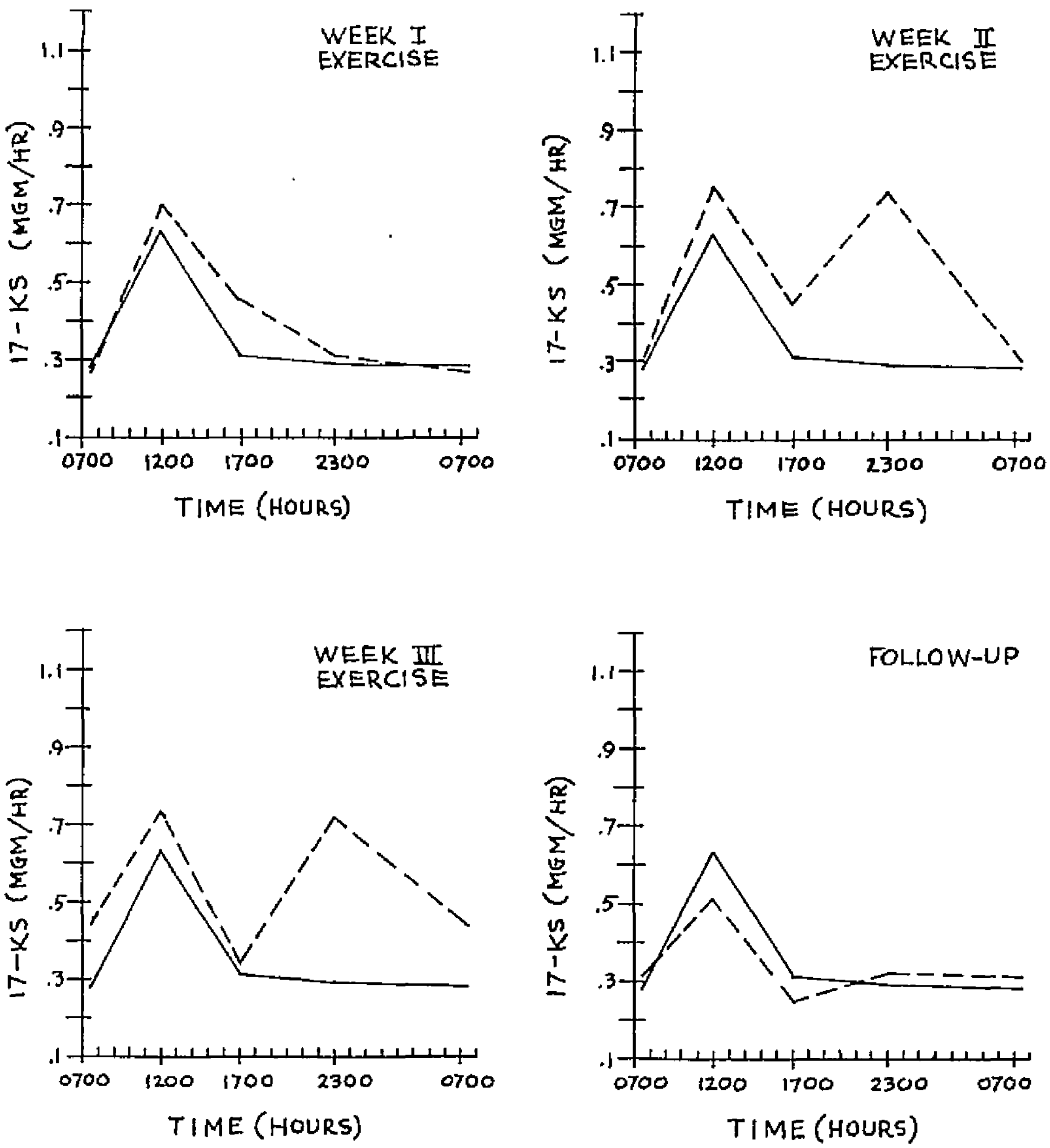
CIRCADIAN VARIATION IN 17-KS VALUES
BEFORE, DURING, AND AFTER A THREE WEEK
TRAINING PROGRAM FOR SUBJECT D



Control: ———— Experimental: - - - -

FIGURE 5

CIRCADIAN VARIATION IN 17-KS VALUES
BEFORE, DURING AND AFTER A THREE WEEK
TRAINING PROGRAM FOR SUBJECT E



Control: ——— Experimental: - - - -

FIGURE 6

CIRCADIAN VARIATION IN 17-KS VALUES
BEFORE, DURING, AND AFTER A THREE WEEK
TRAINING PROGRAM FOR SUBJECT F

program were parallel to the control values for the subjects in the study. The 17-KS values were elevated above the control values for all the subjects except subject F. For subject F the 1200 and 1700 hours values were below the control values.

RELATIONSHIP BETWEEN PHYSICAL PERFORMANCE AND ADRENAL CORTICAL CIRCADIAN RHYTHM

Prior to the three week training period a comparison was made between duration of exercise scores from 0900 to 1100 hours and 2100 to 2300 hours to determine if peak performance was related to peak adrenal cortical function. Following the three week training period the procedure was repeated to determine if any alteration in the adrenal cortical circadian rhythm influenced physical performance. The duration of exercise scores are presented in Table I.

The control circadian patterns for the six subjects revealed peak 17-KS values from 0730 to 1200 hours. However, only subjects B and E had the greater duration of exercise scores during the 0900 to 1100 hours time period. The other four subjects had the greater duration of exercise scores during the 2100 to 2300 hours time period.

Following the three week training period the adrenal cortical circadian pattern for the six subjects revealed a primary peak and a secondary peak, the primary being at 1200 hours and the secondary being at 2300 hours. The duration of exercise scores for all six subjects were greater at the 0900 to 1100 hours time period. Also, the post-training scores were greater than the pre-training scores for all six subjects.

TABLE I

DURATION OF EXERCISE SCORES BEFORE
AND AFTER A THREE WEEK TRAINING PROGRAM

	Pre-Training Scores (Minutes)			Post-Training Scores (Minutes)		
Subject	0900 to 1100 Hrs	2100 to 2300 Hrs	Difference	0900 to 1100 Hrs	2100 to 2300 Hrs	Difference
A	9:58	10:45	-0:47	12:07	12:00	+0:07
B	7:56	7:52	+0:04	9:38	9:30	+0:08
C	10:51	11:02	-0:11	11:49	11:15	+0:34
D	8:45	8:58	-0:13	10:41	10:28	+0:13
E	7:31	7:27	+0:04	10:24	10:09	+0:15
F	6:58	7:18	-0:20	9:19	9:14	+0:05

+ and - indicate direction of difference relative to 0900-1000 Hrs.

CHAPTER V

SUMMARY
FINDINGS, DISCUSSION, CONCLUSION, AND RECOMMENDATIONS

SUMMARY

The effect of a specific exercise program on the circadian rhythm of the adrenal cortex was studied utilizing six male students as subjects. The concentration of the 17-KS in the urine served as an indicator of adrenal cortical function.

The exercise program consisted of an exercise bout on a bicycle ergometer. These bouts were conducted once a day for five consecutive days per week over a three week period. At each exercise bout the work load on the ergometer was started at zero kilo-pond-meters per minute and increased 150 kilo-pond meters per minute of exercise until the heart rate reached 180 beats per minute.

The circadian pattern for each subject was determined by plotting results of urinanalysis conducted four times during a twenty-four hour period. Two such twenty-four hour determinations were made each week. From these determinations an average was calculated for each week. The week prior to the beginning of the exercise program was used to establish a control pattern.

In order to investigate the effect of the exercise program on the circadian rhythm of adrenal cortical function the circadian pattern for each week of the exercise program was graphically compared with the control pattern for each subject. The circadian pattern for the week following the exercise program was also compared with the control pattern for each subject.

In order to determine the relationship between physical performance and the adrenal cortical circadian rhythm each subject performed an exercise bout on the bicycle ergometer at 0900 hours and at 2100 hours prior to the three week training period. The duration of exercise at each exercise bout was recorded in minutes as the pre-training scores. At the end of the three week training program the same procedure was repeated with the duration of exercise being recorded as post-training scores.

A comparison was made between the two pre-training scores at 0900 hours and 2100 hours to determine if peak performance was related to peak adrenal cortical function. A comparison was also made between post-training scores to determine if an alteration in the adrenal cortical cycle was related to a change in physical performance.

FINDINGS

The findings of this study were as follows:

1. Three basic circadian control patterns were found

among the six subjects. One pattern had a peak value at 1200 hours with the low 17-KS value at 0700 hours. The second pattern had a peak value at 1200 hours with the low value being at 2300 hours. The third pattern also had the primary peak at 1200 hours, a lower secondary peak at 2300 hours and a low value at 0700 hours.

2. As compared to control values, four of the subjects had an increase in 17-KS values during week one of exercise while two subjects had a decrease in 17-KS values.
3. During week two of exercise the 17-KS values for all the subjects increased as compared to the control values. At 2300 hours five of the subjects developed a secondary peak 17-KS value.
4. All of the six subjects revealed a primary and a secondary 17-KS peak during exercise week three.
5. The 17-KS values for the week following the exercise program were, with the exception of one subject, elevated as compared to the control values. The 17-KS circadian patterns were parallel to the control patterns for all six of the subjects.
6. Prior to the training period two subjects had greater duration of exercise scores at the time of peak 17-KS values; however, four subjects had

greater duration of exercise scores during the low point for 17-KS values.

7. Following the three week training program the post-training scores were greater than the pre-training scores for all six subjects.

8. Following the training program the duration of exercise scores for all six subjects were greater at the 0900 to 1100 hours time period than for the 2100 to 2300 hours time period.

DISCUSSION

The relationship of physical training and glandular function was the subject of a review by Cureton.[58] Part of this review dealt with the effect of exercise on the adrenal gland and on the 17-KS output in the urine. In this review it was concluded that physical training improves the function of the adrenal gland for hard work by increasing the corticosteroid output. It was also suggested that endurance-type athletes are able to excrete more 17-KS, a probable indicator of larger adrenal glands.

In this study the effect of an endurance-type exercise program on the circadian rhythm of the adrenal cortical function as measured by urinary 17-KS output was the same

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[58]Thomas K. Cureton, "Physical Training Helps to Regulate and Improve Glandular Function," Research Quarterly, 30:270-273, 1959.

for all six subjects. As compared to control values a secondary 17-KS peak appeared during the third week of exercise. Upon termination of the exercise program this secondary peak disappeared and the circadian pattern returned to control patterns for all subjects. This observation suggested that the exercise program was the causative factor in the alteration of the circadian pattern.

The mechanism by which exercise altered the adrenal cortical circadian pattern was not part of the design for this study; however, the central nervous system-pituitary mechanism suggested by Mills[59] offers a possible explanation. According to Mills, periodic stimuli impinging on the central nervous system results in a rhythmical release of corticotrophic-releasing-factor (CRF) from the region of the median eminence. The rate of synthesis and release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary depends on the action of CRF. This periodic release of ACTH results in rhythmic activity of the adrenal cortex.

In view of this mechanism, any alteration in the adrenal cortical circadian rhythm suggests comparable alteration in the release of CRF by the central nervous system. It is possible that anticipation by the subject of stress imposed by the exercise bout resulted in an increased release of

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[59] J. N. Mills, "Human Circadian Rhythms," Physiological Review, 46:128-171, 1966.

CRF at a time when this release was at a low point.

A hypothesis put forth by Long[60] could offer a possible explanation for alteration in adrenal cortical circadian pattern. According to this scheme increased secretion of epinephrine during stress causes an increased pituitary release of ACTH, with consequent adrenal cortical secretion to meet the needs created by the stress. Thus, in this study, the stress imposed by exercise could result in an increase in adrenal activity when such activity was at a low point. However, according to Mountcastle[61] while this hypothesis by Long is applicable to rats it has not been successfully demonstrated in either dog or man.

The lack of relationship between physical performance and the circadian rhythm of the adrenal cortex could be attributed to the use of heart rate in the determination of duration of exercise scores. As used in this study the duration of exercise scores depended upon the resting heart rate.[62] A high resting heart rate resulted in a short duration of exercise score, whereas, a low resting heart rate resulted in a long duration of exercise score.

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[60] C. N. H. Long, "The Role of Epinephrine in the Secretion of the Adrenal Cortex," Ciba Colloquia Endocrinology, 4:139, 1952.

[61] Mountcastle, loc. cit., pp. 968-969.

[62] K. E. Klein and others, "Circadian Rhythm in Indices of Human Performance, Physical Fitness, and Stress Resistance," Journal of Aerospace Medicine, 39:512-518, 1968.

Heart rate is known to follow a circadian pattern; thus, heart rate has a maximum value in the morning and a minimum during the evening hours.[63] Since heart rate follows a circadian pattern, it was expected that duration of exercise scores would be lower in the morning hours as compared to the evening hours. Four subjects in this study had pre-training duration of exercise scores that were lower in the morning hours as compared to the evening hours.

All six subjects in the study had post-training duration of exercise scores that were greater in the morning hours as compared to the evening hours. Also, all six subjects had post-training duration of exercise scores that were greater than the pre-training duration of exercise scores. Thus, it would seem that the exercise program lowered the resting heart rate and lowered it to a greater degree during the morning hours as compared to the evening hours. This, however, did not indicate a relationship between adrenal cortical circadian function and physical performance.

CONCLUSIONS

Within the limitations of this study the following conclusions were reached:

1. The exercise program utilized in this study

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[63] N. Kleitman and others, "Periodicity in Body Temperature and Heart Rate," Endocrinology, 43:1-20, 1948.

altered the circadian pattern of the adrenal cortex as determined by the urinary 17-KS.

2. There appeared to be no relationship between physical performance ability and the circadian variation in adrenal cortical function as determined by urinary 17-KS neither before nor after the three week training program.

RECOMMENDATION

It was suggested that the following studies be conducted:

1. A study to determine the effect of exercise programs on adrenal cortical circadian function at various times during a twenty-four hour period since this study dealt only with exercise during one interval of time during a twenty-four hour period.
2. A study to determine the effect of a strength training program on the circadian rhythm of the adrenal cortex since this study dealt only with an endurance type exercise program.
3. A study to determine the relationship between heart rate circadian rhythm and adrenal cortical circadian pattern.

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APPENDIXES

APPENDIX A

17-KETOSTEROID VALUES FOR SUBJECT A

		Time				
Week	Sample	0730	1200	1700	2300	24-Hour Total
	1	.15	.38	.28	.22	
1	2	.13	.34	.24	.19	
	Average	.14	.36	.26	.21	5.37
	1	.20	.39	.37	.22	
2	2	.21	.42	.39	.28	
	Average	.21	.41	.38	.25	7.04
	1	.21	.48	.36	.41	
3	2	.20	.54	.40	.50	
	Average	.21	.51	.38	.46	8.74
	1	.23	.55	.33	.52	
4	2	.24	.57	.37	.53	
	Average	.24	.56	.35	.53	9.49
	1	.22	.43	.30	.27	
5	2	.19	.40	.31	.29	
	Average	.21	.42	.31	.28	6.91

APPENDIX B

17-KETOSTEROID VALUES FOR SUBJECT B

		Time				
Week	Sample	0730	1200	1700	2300	24-Hour Total
1	1	.22	.56	.28	.11	
	2	.24	.52	.20	.15	
	Average	.23	.54	.24	.13	6.36
2	1	.20	.53	.31	.19	
	2	.23	.58	.30	.17	
	Average	.22	.56	.31	.18	7.02
3	1	.31	.64	.33	.39	
	2	.29	.70	.31	.44	
	Average	.30	.67	.32	.42	9.69
4	1	.28	.59	.30	.51	
	2	.30	.63	.38	.55	
	Average	.29	.61	.34	.53	10.08
5	1	.24	.62	.30	.28	
	2	.21	.56	.31	.18	
	Average	.23	.59	.31	.23	7.53

APPENDIX C

17-KETOSTEROID VALUES FOR SUBJECT C

Week	Sample	Time 0730	Time 1200	Time 1700	Time 2300	24-Hour Total
1	1	.12	.62	.29	.43	
	2	.18	.65	.30	.38	
	Average	.15	.64	.30	.41	8.12
2	1	.16	.65	.45	.39	
	2	.23	.59	.52	.44	
	Average	.20	.62	.49	.42	9.46
3	1	.29	.70	.50	.62	
	2	.31	.72	.61	.70	
	Average	.30	.71	.56	.66	12.51
4	1	.25	1.09	.44	.94	
	2	.32	.89	.49	.84	
	Average	.29	.99	.47	.89	14.60
5	1	.28	.70	.38	.50	
	2	.20	.71	.35	.49	
	Average	.24	.71	.37	.50	10.09

APPENDIX D

17-KETOSTEROID VALUES FOR SUBJECT D

		Time				
Week	Sample	0730	1200	1700	2300	24-Hour Total
1	1	.15	.60	.33	.38	
	2	.25	.65	.30	.32	
	Average	.20	.63	.32	.35	8.23
2	1	.33	.48	.25	.24	
	2	.18	.59	.15	.30	
	Average	.26	.54	.20	.27	7.26
3	1	.29	.48	.43	.44	
	2	.32	.38	.31	.36	
	Average	.31	.43	.37	.40	8.83
4	1	.49	.53	.19	.40	
	2	.42	.49	.29	.41	
	Average	.46	.51	.24	.41	9.86
5	1	.27	.71	.39	.41	
	2	.32	.60	.33	.35	
	Average	.30	.66	.36	.38	9.60

APPENDIX E

17-KETOSTEROID VALUES FOR SUBJECT E

Week	Sample	Time: 0730	1200	1700	2300	24-Hour Total
1	1	.28	1.00	.50	.34	
	2	.30	1.08	.64	.37	
	Average	.29	1.04	.57	.36	12.15
2	1	.25	.98	.24	.38	
	2	.29	.88	.24	.50	
	Average	.27	.93	.24	.44	10.32
3	1	.40	1.15	.81	.74	
	2	.49	.99	.91	.64	
	Average	.45	1.07	.86	.69	17.07
4	1	.57	1.07	.84	.97	
	2	.44	1.11	.80	.89	
	Average	.51	1.09	.82	.95	19.03
5	1	.51	1.10	.71	.62	
	2	.39	1.02	.58	.51	
	Average	.45	1.04	.65	.57	15.37

APPENDIX F

17-KETOSTEROID VALUES FOR SUBJECT F

Week	Sample	Time 0730	1200	1700	2300	24-Hour Total
1	1	.30	.62	.31	.30	
	2	.26	.64	.31	.27	
	Average	.28	.63	.31	.29	8.51
2	1	.25	.60	.50	.34	
	2	.29	.79	.42	.29	
	Average	.27	.70	.46	.31	9.60
3	1	.26	.84	.48	.77	
	2	.34	.69	.42	.70	
	Average	.30	.75	.45	.74	11.63
4	1	.41	.66	.43	.63	
	2	.46	.79	.24	.81	
	Average	.44	.73	.34	.72	13.05
5	1	.34	.61	.27	.29	
	2	.28	.40	.22	.34	
	Average	.31	.51	.25	.32	8.11

VITA

The author was born in Natchitoches, Louisiana, and received his elementary and high school education in Many, Louisiana.

The Bachelor of Science degree in Liberal Arts with a major in mathematics was awarded in 1961 by Louisiana Polytechnic Institute, Ruston, Louisiana.

Until 1963 the author was employed at various capacities in the petroleum industry. In September of 1963 he entered the Louisiana State University School of Medicine.

In 1965 the author was employed as mathematics and science instructor, head track coach, and assistant football coach at Many High School, Sabine Parish, Louisiana.

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EXAMINATION AND THESIS REPORT

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Major Field: Physical Education

Title of Thesis: The Effect of a Specific Exercise Program on the Circadian Rhythm of the Adrenal Cortex as Determined by the Urinary 17-Ketosteroids

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July 19, 1971